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Research Article

Characterization of Pathogen-specific Metabolites by Thin-film Solid Phase Microextraction Analytical Tool

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Abstract

Respiratory infection caused by the bacterial species is a leading cause of morbidity and mortality globally, especially in immunocompromised people. All available diagnostic techniques for pneumonia detection are relatively limited due to time-consuming stages, selectivity, and the need for invasive procedures. To overcome the current challenges, we developed an analytical technique, utilizing a carbon mesh-supported thin film solid-phase microextraction (TF-SPME) technique coupled to gas chromatography-mass spectrometry for monitoring of pathogen-specific metabolites from bacterial culture media of *E. coli* bacterial strain. We exposed the TF-SPME device from the headspace of the culture media of the bacteria and extracted the metabolites during the growth phase of the *E. coli*. TF-SPME was thermally desorbed in GC-MS to identify the compounds. We observed the pathogen-emitted metabolites, including Pentane, 3-methyl (RT 1.645), Cyclopentane, methyl (RT 1.876), Cyclohexane (RT 2.099), 2-pentanone (RT 2.3), Benzene, 1-ethyl-4-methyl (RT 5.673), and Cyclopropane, 1, 1-dichloro-3 (1, 1-dimethyl ethyl)-2, 2-dimethyl (RT 12.525) in culture media. This technique may be helpful for the non-invasive diagnosis of bacterial pathogens without culture studies.

Introduction

Ventilator-associated pneumonia (VAP) is characterized as a nosocomial infection that occurs extensively in ventilated patients at the intensive care unit (ICU) after the 48-hour intubation period.[1] It is characterized by clinical symptoms such as sudden onset of fever, elevated WBC count (leukocytosis), purulent sputum, and the presence of positive culture from the sputum or the trachea.[2] The traditional method of screening VAP involves three significant criteria: clinical, radiographic, and laboratory indicators. However, these methods cannot directly diagnose the disease.[3] The conventional method involves the collection of Bronchoalveolar Lavage (BAL) or Protected Specimen Brush (PSB) for the quantitative analysis of the microbiological aspects through invasive diagnosis. Another approach involves deploying bronchoscopy, which is non-invasive and associated with risks such as minimal complication, cost, and complicated process.[4] However, it is a very time-consuming process that can take up to two days to diagnose and between one and two days to test susceptibility to antibiotics. Conventional microbiological diagnostics usually take an average of 71 hours from the sample to finalize the results.[5] The challenges of managing VAP recapitulate the absence of a reliable technique, lack of prophylactic measures, and the risk associated with antibiotic resistance. Some studies show that one of the main reasons for the unnecessary prescription of antibiotics in ICU is a lack of early disease diagnosis, with estimates

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ranging from 30% to 60%.[6] ICU infection is an independent predictor for poor prognosis, and VAP caused by multidrug-resistant microbes and extensively drug-resistant strains is still very challenging due to its ability to evolve rapidly and the treatment becoming very difficult, complementing increasing cost.[7] Gram-negative bacteria are found to be the primary source of ICU infections worldwide.[8] Escherichia coli (E. coli) is a common bacterium that can cause VAP in adult ICU patients.[9] Studies have shown that *E. coli* isolates responsible for VAP exhibit antibiotic resistance and certain genotypic characteristics. It has been found that *E. coli* causes pneumonia and has a higher virulence factor gene content and antimicrobial drug resistance.[10] Several techniques are available in contemporary medicine to diagnose pneumonia. However, none of the methods are reliable, and the interpretation may vary among each individual while analyzing the results. The thin film solid phase microextraction (TF-SPME) is an analytical technique for extracting the trace levels of volatile and semi volatile compounds from the sample matrices.[11] It is coated with polydimethylsiloxane (PDMS) coated with either Carboxen (CAR), Divinylbenzene (DVB), or Hydrophilic-Lipophilic Balance (HLB particles). The planar structure and increasing the surface area of TF-SPME made it possible to reduce the extraction time without compromising the extraction rate. No study has been done on the headspace analysis of bacterial pneumonia using the TF-SPME membrane. Here, we have investigated the bacteria-produced metabolites using the TF-SPME device to monitor volatile metabolites during the growth phase of *E. coli* in culture media. The study demonstrated the feasibility of monitoring bacterial volatile metabolites for the characterization of pathogens.

Materials and Methods

Chemicals and Instrumentation

TF-SPME membrane coated with PDMS/HLB was purchased from Markes International, UK. Apart from this, we utilized the Luria-Bertani Broth, Nutrient Agar, Acetonitrile (ACN), Conical flask, Supelco vial, and regular laboratory consumables for the study. TF-SPME Membranes were thermally desorbed in the Gas Chromatography-Mass spectrometry (Shimadzu, Japan) instrument present in KMC Hospital, Manipal.

Preparation of bacterial culture

The experiment utilized the bacterial glycerol stocks of *E. coli* to produce a single, well-isolated colony onto nutrient agar media. The culture plates were incubated at 37^0C for 24 hours. The obtained colony was inoculated in Luria-Bertani broth, and then we kept it in a shaking incubator until the OD value of the bacterial solution reached from 0.1 to 0.5. We extracted the bacterial metabolites at the growth phase of culture media.

Determination of pathogen-specific metabolites

Humans naturally release volatile organic compounds (VOCs) in normal metabolism, some of which can be detected even at a sparse level. The chemical patch incorporating nanomaterials blended with Hydrophilic-Lipophilic Balance (HLB) particles was utilized for the study of extracting hydrophilic, lipophilic, and hydrophobic compounds from culture media. This tool enabled us to extract the polar and non-polar, semi-volatile, and very volatile compounds. To obtain the pathogen-specific metabolites from *E. coli*, the TF-SPME membrane was exposed to the headspace of the 20 ml of culture media during the bacterial growth for 1 hour and 30 minutes in the flask. The membranes were then carefully removed and dissolved in 1 ml of acetonitrile to extract the metabolites captured by the TFME membrane (Figure 1).

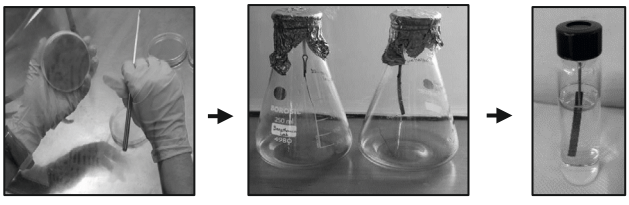

Figure 1: Methodology for monitoring the pathogen-specific metabolites by TF-SPME device

We desorbed the TF-SPME membrane in GC-MS (Shimadzu) with the following program run: the temperature was initially held at 40^0C for 2 min,

ramped at a rate of 20^0C s^1 to 280^0C (Figure 2). We used the DB-5 column for this purpose.

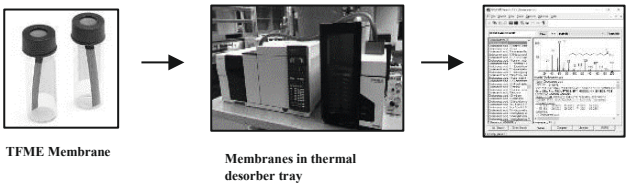

Figure 2: Desorption of TF-SPME membrane in GC-MS and identification of compounds by NIST library

Results

The TF-SPME device was exposed to the headspace of the culture media in a closed conical flask until the OD value of the culture media reached 0.5 from 0.1. After 1 hr of incubation of bacterial media exposed to headspace TFME, the membrane was carefully removed and dissolved in 1 ml of acetonitrile and sent for GC-MS analysis. The obtained graphs for blank and culture media were analyzed to obtain the metabolites released from the bacteria. Figure 3 depicts the chromatogram obtained from the cultured *E. coli* media analysis when analyzed using NIST Library, Mass Hunter. The obtained metabolites include Pentane, 3-methyl (1.645), Cyclopentane, methyl- (1.876), Cyclohexane (2.099), 2-Pentanone (2.3), Benzene, 1-ethyl-4-methyl (5.673) and Cyclopropane,1, 1-dichloro-3(1, 1-dimethylethyl)-2, 2-dimethyl (12.525).

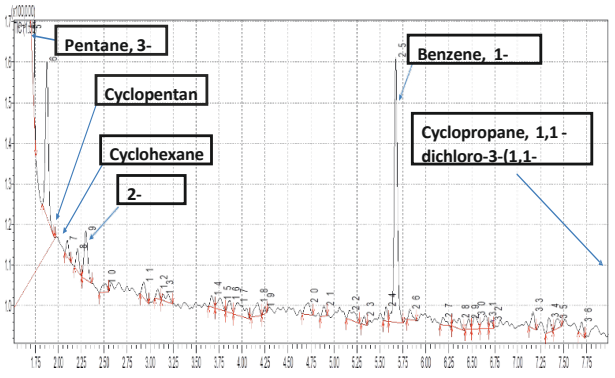

Figure 3: Chromatogram of *E. coli*-emitted metabolites from culture media by TF-SPME device

Conclusion

With a population of 1.3 billion people and most people living in rural regions, it might be difficult for our government to provide the necessary healthcare facilities everywhere for the sake of the ordinary people. This research proposes developing an analytical technique that may provide a potential non-invasive platform for rapid assessment of *E. coli* infection in individuals with respiratory infection. Furthermore, this technology can shorten the diagnosis times and save the lives of millions of cancer patients who are dying from pneumonia because of mandatory waiting time for pathology reports. Therefore, further developing this technique may offer a practical solution to one of our society's most prevalent health challenges. More studies are required to confirm the metabolic profiles of the bacterial species.

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